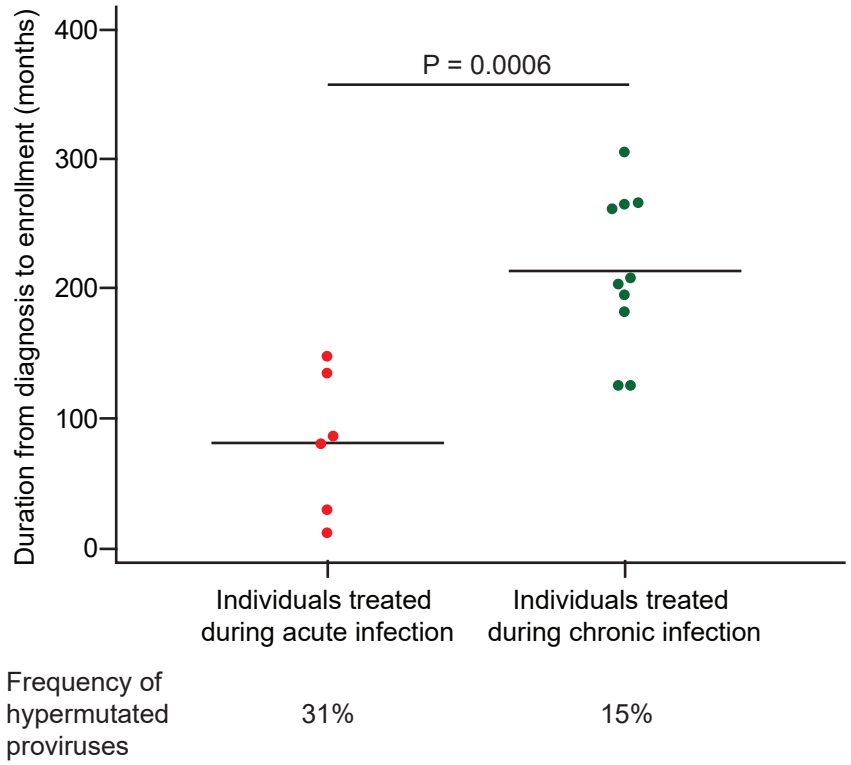


Figure S1. The duration of infection may contribute to the different frequency of hypermutated proviruses in individuals treated during acute and chronic infection. The P value was calculated by two-tailed Student *t*-test. Associated with Figure 1.

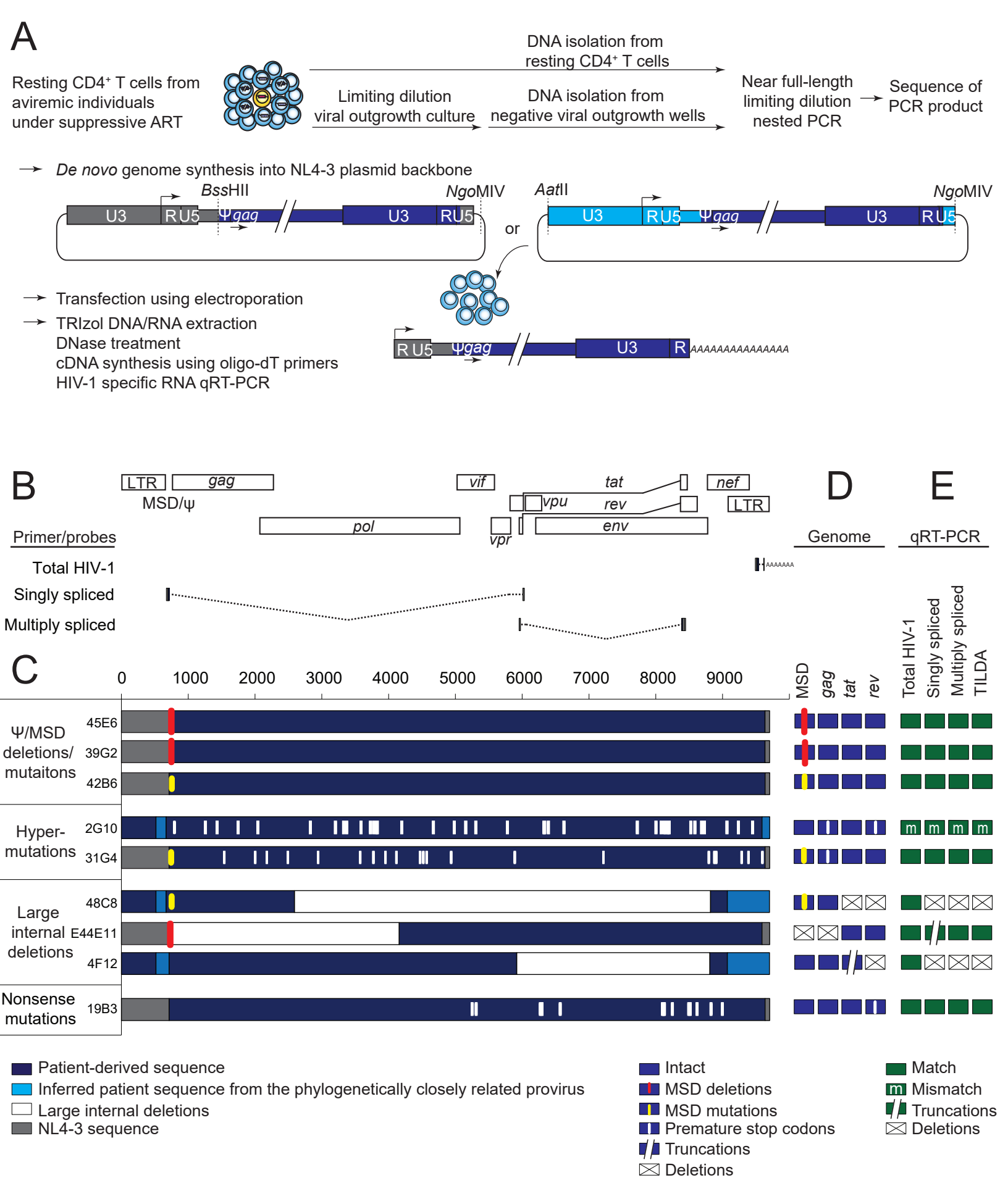


Figure S2. Map of reconstructed patient-derived defective HIV-1 proviruses. (**A**) Experimental scheme for isolation and reconstruction of patient-derived defective HIV-1 proviruses. (**B**) HIV-1 genome map and qRT-PCR primer binding sites. (**C**) Maps of reconstructed defective proviruses. (**D**) Status of HIV-1 genetic elements. (**E**) Status of qRT-PCR primer/probe binding sites. Associated with Figure 2.

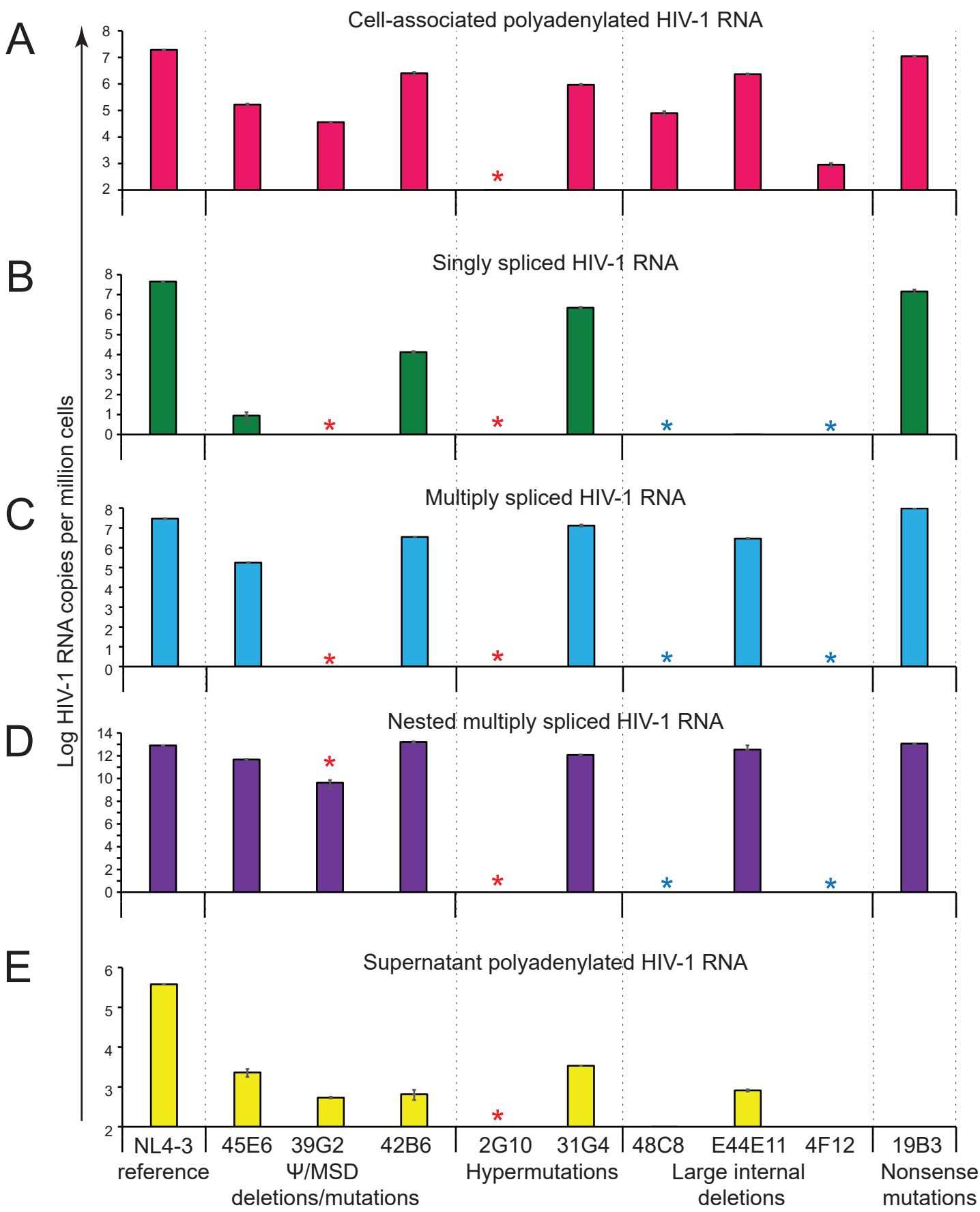
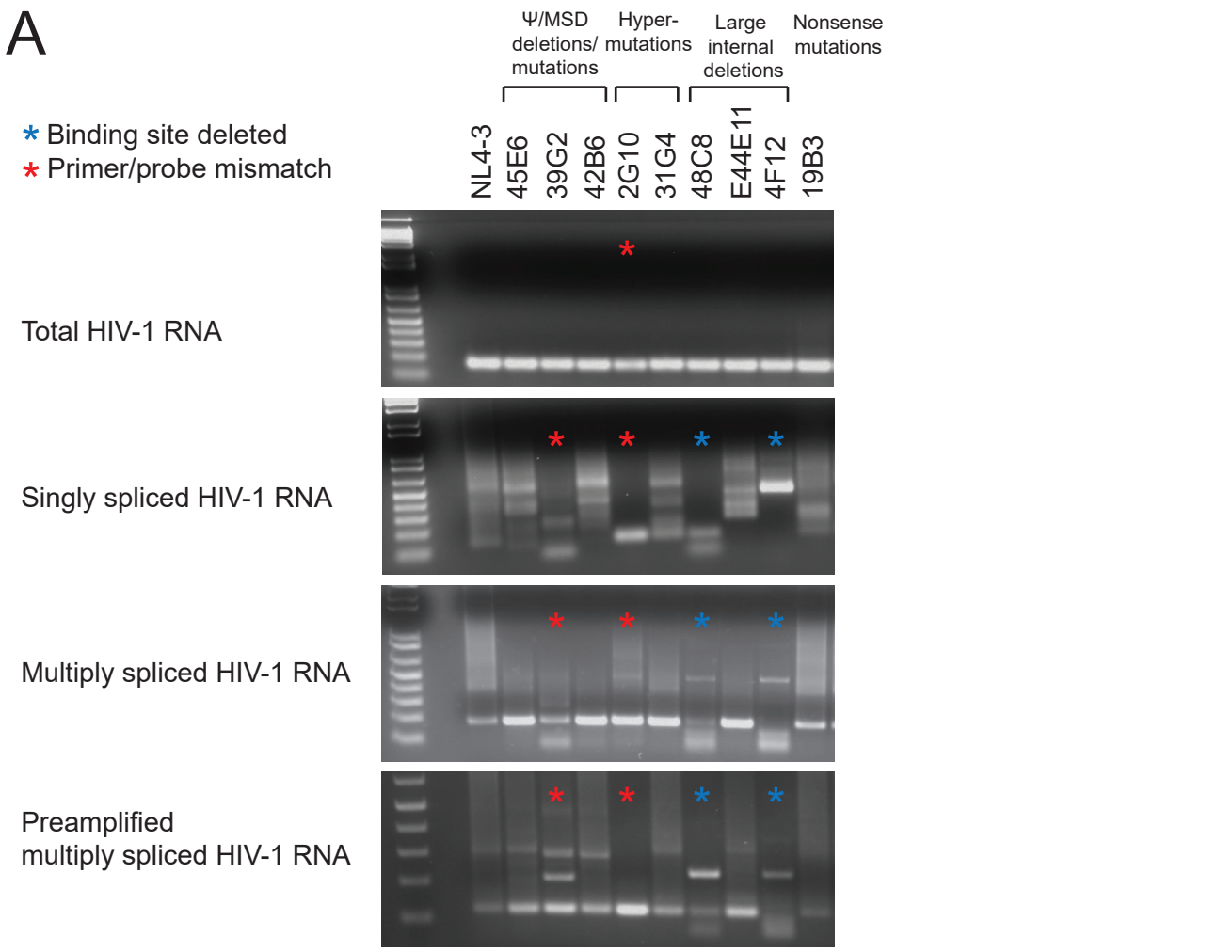


Figure S3. Defective HIV-1 proviruses can be transcribed *in vitro*. Activated primary CD4$^+$ T cells were transfected with reconstructed patient-derived HIV-1 proviral plasmids. HIV-1 RNA was converted to cDNA using oligo-dT-based priming after DNase treatment. Levels of cell-associated polyadenylated HIV-1 RNA (**A**), singly spliced (**B**), multiply spliced (**C**) and preamplified multiply spliced HIV-1 RNA (**D**) in transfected activated primary CD4$^+$ T cells were measured by qRT-PCR. HIV-1 RNA quantity was normalized to the number of cells as measured by RNaseP qPCR in the corresponding DNA component of the transfected cells. (**E**) Levels of supernatant HIV-1 RNA was measured by poly-adenylated HIV-1 RNA quantification. Culture supernatant was treated with RNaseA to remove virion-free RNA. Data represent mean ± SEM. Associated with Figure 2.



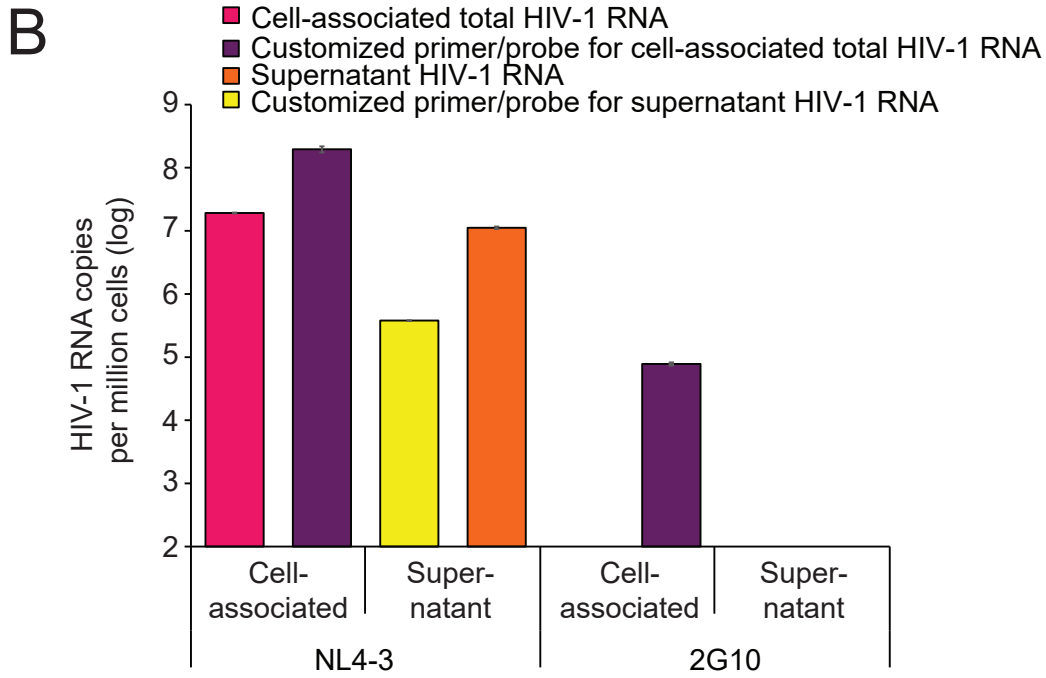


Figure S4. Hypermutated HIV-1 proviruses can be transcribed *in vitro* despite a mutated LTR promoter.
(**A**) Agarose gel electrophoresis (1.5%) of qRT-PCR products. Note that some clones which has sequence mismatch with the qPCR amplification probes (yellow asterisks) can be detected by gel electrophoresis. (**B**) Cell-associated and supernatant HIV-1 RNA level of 2G10 using customized qRT-PCR primer/probe to match the G→A hypermutations. Data represent mean ± SEM. (**C**) Sequence of NF-κB and Sp1 binding sites in HIV-1 LTR. Red, G→A hypermutations. Associated with Figure 2.

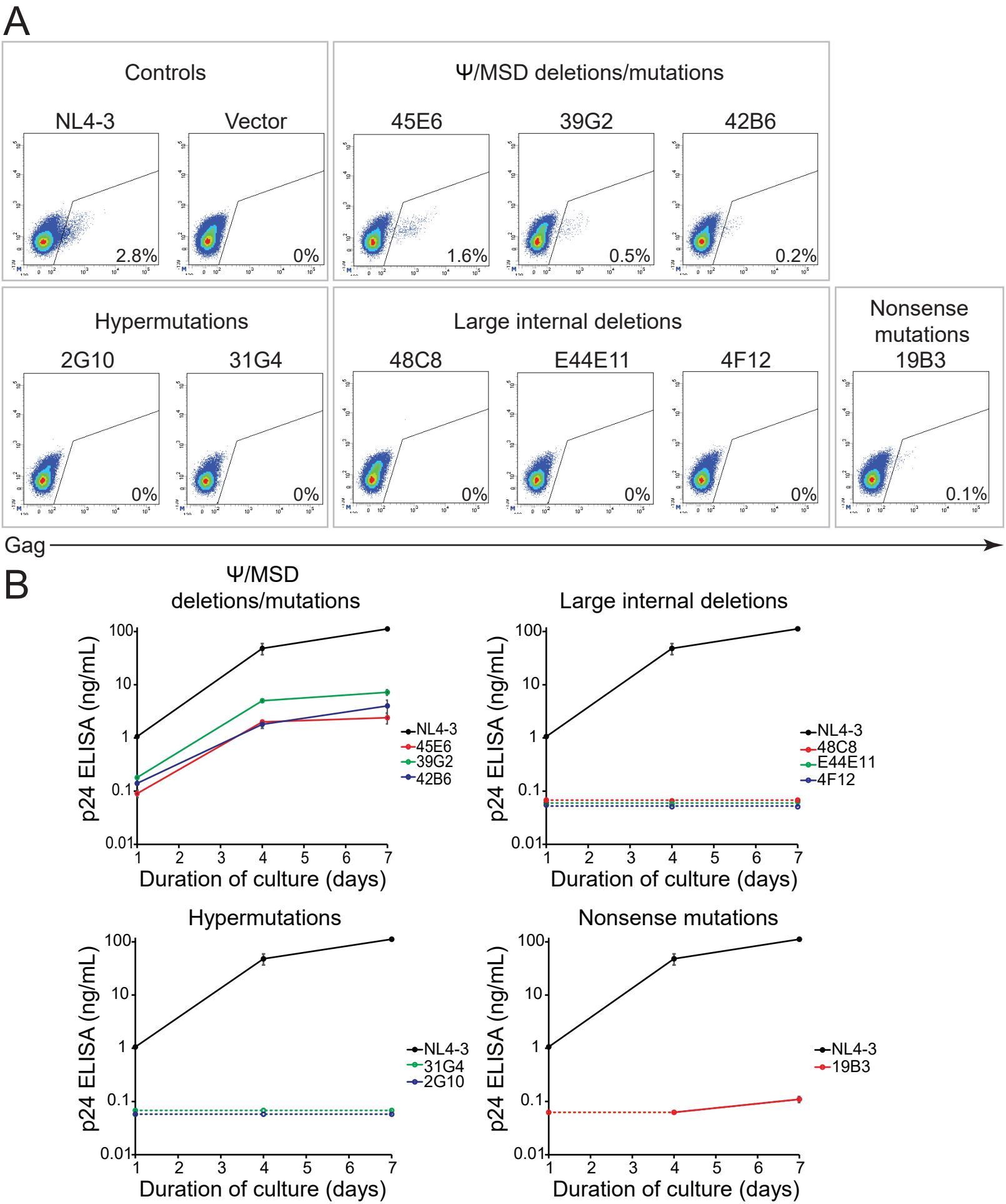


Figure S5. Defective HIV-1 proviruses can be translated *in vitro*. (**A**) Intracellular Gag staining (**B**) supernatant p24 ELISA of primary activated CD4$^+$ T cells transfected with defective patient-derived proviral plasmids in the presence of enfuvirtide. Data represent mean ± SEM. Open circles and dotted lines indicate limit of detection. Asociated with Figure 2.

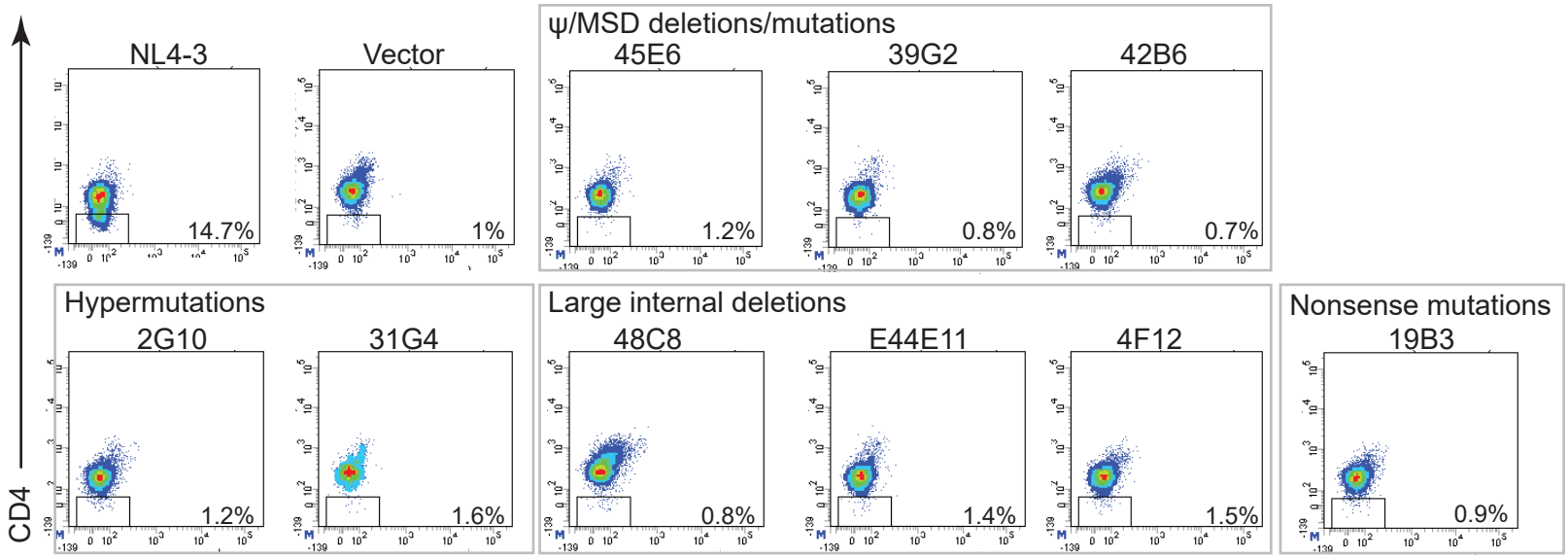
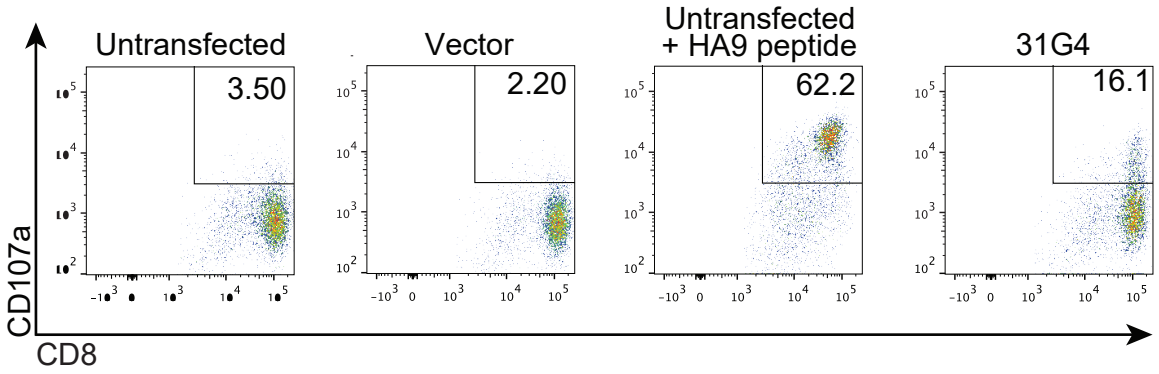


Figure S6. Defective HIV-1 proviruses cannot downregulate CD4 expression *in vitro*. CD4 expression level in primary activated $CD4^+$ T cells transfected with defective patient-derived proviral plasmids in the presence of enfuvirtide. Associated with Figure 2.

A



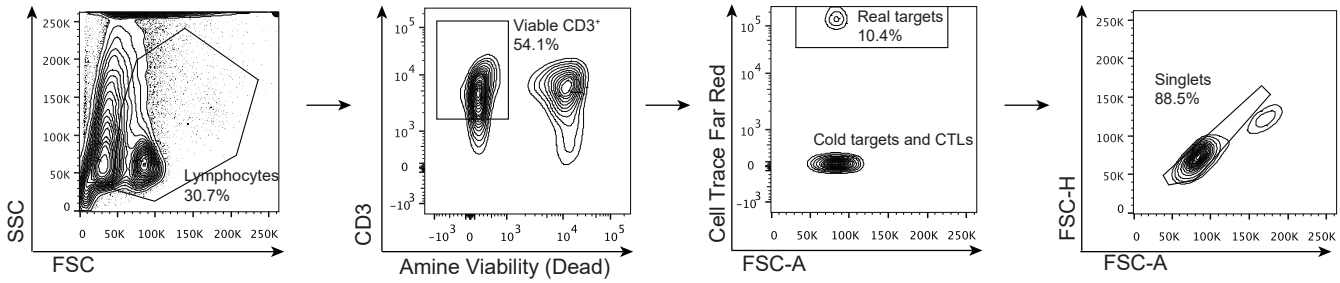
B

	Effector CTL	Real targets	Cold targets
	B27-Gag-IK9 specific CTL clone	Autologous CD4⁺ T cells infected with HIV-1_{LAI} and stained with Cell Trace Far Red	Autologous CD4⁺ T cells transfected with defective proviruses
CTL: real target : cold target (input cell number)	1 :	20 :	800
	1 :	100 :	4000
Real target: cold target (input cell number)		1 :	40
Estimated proportion of infected/transfected cells		~10%	<3%
Estimated infected real target: transfected cold target		1 :	<12

%Infected real targets = $\dfrac{\text{Gag}^{+}\text{CD4}_{dim} \text{ real target cells}}{\text{CellTrace Far Red stained cells}}$

%Infected targets killed = $\dfrac{\text{\%Infected real targets in no CTL sample} - \text{\%Infected real targets in test sample}}{\text{\%Infected real targets in no CTL sample}}$

C



D

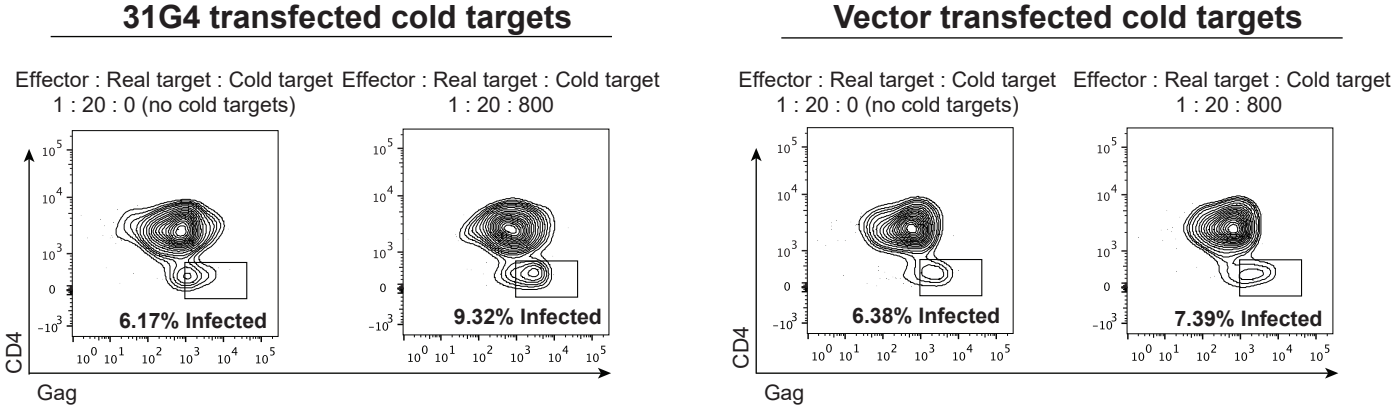


Figure S7. Experimental scheme of CTL recognition *in vitro*. (A) Representative flow cytometry plots for CD107a expression. (**B**) Experimental scheme for cold-target inhibition. (**C**–**D**) Representative flow cytometry plots. $Gag^{+}CD4^{low}$ cells were gated to identify productively infected cells instead of cells containing incoming Gag without active viral protein production ($Gag^{+}CD4^{high}$). Gating was guided by the staining of negative controls. Associated with Figure 4 and 5.

Table S1. Demographics of study participants

Participant ID	Age	Gender	Ethnicity[a]	ART regimen[b]	Duration of viral load <50 copies/ml (months)	Viral load (copies/mL)	Note
Defective HIV-1 provirus reconstruction							
YA9	57	M	AA	FTC/TDF, FPV/r	28	<50	31G4
YA10	47	M	W	FTC/TDF, FPV/r	60	<50	45E6, 48C8
YA17	49	M	AA	3TC/AZT, EFV, ATV/r	57	<50	2G10, 4F12
YA19	46	F	AA	ABC/3TC, NVP	105	<50	42B6, 19B3
YA22	38	M	AA	ABC/3TC, EFV	52	<50	39G2
CP03	76	M	W	ABC/3TC, ATV/r	191	<50	E44E11
Ex vivo HIV-1 transcription							
78	60	M	W	EFV/FTC/TDF	131	<50	
79	65	F	AA	FTC/RPV/TDF	119	<50	
80	51	M	W	FTC/TDF, ATV/r	63	<50	
81	56	M	W	EFV/FTC/TDF, RAL	120	<50	
84	62	F	AA	ABC/3TC, ATV/r	53	<50	
188	59	F	AA	FTC, ETV, RAL	66	<50	
216	60	M	W	3TC, DTG, DRV/r	155	<50	
382	64	F	AA	ABC/3TC/DTG	44	<50	
CTL clones							
OM5011	44	M	W	ABC/3TC, DTG	77	<50	
OM5267	25	M	W	ABC/3TC, RAL	25	<50	

[a]AA, African-American; W, White.

[b]Antiretroviral therapy (ART) abbreviations: tenofovir disoproxil fumarate (TDF), emtricitabine (FTC), zidovudine (AZT), lamivudine (3TC), abacavir (ABC), nevirapine (NVP), efavirenz (EFV), etravirine (ETR), rilpivirine (RPV), dolutegravir (DTG), raltegravir (RAL), atazanavir (ATV), fosamprenavir (FPV), darunavir (DRV), ritonavir-boosted (r)

*Associated with Figure 2

Table S2. Splice sites of reconstructed patient-derived HIV-1 proviral plasmids*

		Splice sites											
		Splice donor			Splice acceptor for *vif*	Splice acceptor for *vpr*	Splice acceptor for *tat*	Splice acceptor for *rev and vpu-env*			Splice acceptor for *vpu-env*	Completely spliced RNA for *tat, rev, nef*	
		D1 (MSD)	D1b	D1c	A1	A2	A3	A4a	A4b	A4c	A5	D4	A7
Reference	NL43	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present
Ψ/MSD deletions/mutations (otherwise intact genome)	45E6	Absent	Present	Absent	Present	Present	Present	Present	Present	Present	Present	Present	Present
	39G2	Absent	Present	Absent	Present	Present	Present	Present	Present	Present	Present	Present	Present
	42B6	Mutated (TG|GT→TG|GG)	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present
Hypermutations	2G10	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present
	31G4	Mutated (TG|GT→TA|GT)	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present
Large internal deletions	48C8	Mutated (TG|GT→CG|GT)	Present	Present	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
	E44E11	Absent	Absent	Absent	Present	Present	Present	Present	Present	Present	Present	Present	Present
	4F12	Present	Present	Present	Present	Present	Present	Absent	Absent	Absent	Absent	Absent	Absent
Nonsense mutations	19B3	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present

Table S3. Genomic defects of reconstructed patient-derived HIV-1 proviral plasmids*

		Viral proteins									RRE
		Gag	Pol	Tat	Rev	Env	Nef	Vif	Vpr	Vpu	
Reference	NL4-3	Intact	Intact	Intact	Intact	Intact	Intact	Intact	Intact	Intact	Present
Ψ/MSD deletions/mutations (otherwise intact genome)	45E6	Intact	Intact	Intact	Intact	Intact	Intact	Intact	Intact	Intact	Present
	39G2	Intact	Intact	Intact	Intact	Intact	Intact	Intact	Intact	Intact	Present
	42B6	Intact	Intact	Intact	Intact	Intact	Intact	Intact	Intact	Intact	Present
Hypermutations	2G10	Missense start codon and premature stop codon (aa82)	Premature stop (aa243)	Intact	Premature stop (aa45)	Premature stop (aa55)	Premature stop (aa57)	Premature stop (aa21)	Premature stop (aa54)	Intact	Present
	31G4	Missense start codon and premature stop codon (aa155)	Premature stop (aa34)	Intact	Intact	Premature stop (aa15)	Premature stop (aa57)	Premature stop (aa21)	Premature stop (aa54)	Intact	Present
Large internal deletions	48C8	Intact	Premature stop due to frameshift mutation (aa34)	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
	E44E11	Absent	Truncated (N-terminus to aa693)	Intact	Intact	Intact	Intact	Intact	Intact	Intact	Present
	4F12	Intact	Intact	Truncated (aa31)	Absent	Absent	Absent	Intact	Intact	Absent	Absent
Nonsense mutations	19B3	Intact	Intact	Intact	Premature stop (aa58)	Premature stop (aa15)	Missense start codon and premature stop (aa57)	Premature stop (aa70)	Intact	Intact	Present

Table S4. Primer/probe mismatches of reconstructed patient-derived HIV-1 proviral plasmids*

		PCR Primer/probe mismatches												
		Total (polyA) HIV-1 RNA			Singly spliced (MSD – *tat* 1st exon)			Multiply spliced (*tat* 1st – 2nd exon)			Preamplified multiply spliced (*tat* 1st – 2nd exon)			
		Forward	Reverse	Probe	Forward	Reverse	Probe	Forward	Reverse	Probe	Tat 1.4	Tat2	Rev	Probe
Reference	NL4-3	0/23	0/10	0/20	0/23	0/23	0/20	0/23	0/27	0/18	0/19	1/34	0/27	0/23
Ψ/MSD deletions/mutations (otherwise intact genome)	45E6	1/23	0/10	0/20	1/23	2/23	1/20	1/23	0/27	0/18	0/19	3/34	0/27	0/23
	39G2	0/23	0/10	0/20	1/23	4/23	0/20	1/23	4/27	0/18	1/19	4/34	1/27	4/24
	42B6	1/23	0/10	0/20	0/23	1/23	1/20	0/23	0/27	0/18	0/16	0/34	0/27	0/23
Hypermutations	2G10	0/23	2/10	5/20	0/23	7/23	1/20	1/23	3/27	4/18	0/19	9/34	3/27	6/23
	31G4	0/23	0/10	1/20	0/23	1/23	1/20	0/23	1/27	0/18	0/19	2/34	0/27	1/23
Large internal deletions	48C8	2/23	0/10	0/20	1/23	Absent	0/20	Absent	Absent	Absent	Absent	Absent	Absent	Absent
	E44E11	0/23	0/10	1/20	0/23	3/23	5 bp truncated	0/23	0/27	0/18	0/18	2/34	0/27	0/23
	4F12	0/23	2/10	2/20	0/23	Absent	1/20	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Nonsense mutations	19B3	1/23	0/10	1/20	0/0	1/23	2/20	0/23	0/27	0/18	0/19	0/34	0/27	1/34

*Associated with Figure 2.

Table S5. Primer and probe sequences

Primer/probe	Sequence
Total HIV-1 RNA (Bullen et al., 2014; Shan et al., 2013)	
VQA-F P9501	CAGATGCTGCATATAAGCAGCTG
VQA-R 10T20	TTTTTTTTTTTTTTTTTTTTTGAAGCACTC
VQA-Probe	/56-FAM/CCT GTA CTG /ZEN/GGT CTC TCT GG/3IABkFQ/
Singly spliced HIV-1 RNA (major splice donor site – *tat* first exon)(Shan et al., 2013)	
SS-F P675	GAGGAGATCTCTCGACGCAGGAC
SS-R PTat	GAGAAGCTTGATGAGTCTGACTG
SS-Probe	/56-FAM/CTTGCTGAA/ZEN/GCGCGCACGGC/3IABkFQ/
Multiply spliced HIV-1 RNA (*tat* first – second exon) (Massanella et al., 2015)	
msTat-Rev-F	CTTAGGCATCTCCTATGGAGGA
msTat-Rev-R	GGATCTGTCTCTGTCTCTCTCTCCACC
msTat-Rev-Probe	/56-FAM/CTCTCCACC/ZEN/AGGGGACCCGACAGGCCC/3IABkFQ/
TILDA (*Tat/rev* Induced Limiting Dilution Assay) (Procopio et al., 2015)	
Outer	
tat1.4	TGGCAGGAAGAAGCGGAGA
rev	GGATCTGTCTCTGTCTCTCTCTCCACC
Inner	
tat2	ACAGTCAGACTCATCAAGTTTCTCTATCAAAGCA
rev	GGATCTGTCTCTGTCTCTCTCTCCACC
ProbeHIV	/56-FAM/TTCCTTCGG/ZEN/GCCTGTCGGGTCCC/3IABkFQ/
HIV-1 *gag* (Palmer et al., 2003)	
SCA-F 6F	CATGTTTTCAGCATTATCAGAAGGA
SCA-R 84R	TGCTTGATGTCCCCCCACT
SCA-Probe	/56-FAM/CCACCCCAC/ZEN/AAGATTTAAACACCATGCT/3IABkFQ/
Custom primer for 2G10 hypermutated clone	
2G10-VQA-F	GACCAGATMYGAGCCTRGGA
2G10-VQA-R	TTTTTTTTTTTTTTTTTTTTTTTRRWGCA
2G10-VQA-Probe	/56-FAM/ADCTARGGA/ZEN/ACCCACTGCTTAAGC/3IABkFQ/
Targeted deep sequencing of *gag*	
Outer	
YH_GagOut_F	CCGAACAGGGACCTGAAAGCGAAAG
YH_GagOut_R	TCTTTATCTAAGGGAACTGAAAAATATGCA
Inner	
YH_GagIn_F	(10 bp barcode)GACTAGCGGAGGCTAGAAGGAGAGAG
YH_FullGagIn_R	CTGTATCATCTGCTCCTGTATCTAATAGAGC

*Associated with Figure 2.